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Phytochemical studies of the southern Australian marine alga, *Laurencia elata*

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ABSTRACT

Chemical profiling of the southern Australian marine alga *Laurencia elata* (Rhodomelaceae) employing on-flow and stop-flow HPLC–NMR methodology followed by off-line chemical investigations resulted in the isolation of two C_{16} chamigrenes, cycloelatanene A and B together with three previously reported sesquiterpenes, (3*Z*)-chlorofucin, pacifenol and elatenyne. The chemical structures were elucidated via detailed spectroscopic analyses.

1. Introduction

Red algae belonging to the genus *Laurencia* (Ceramiales, Rhodomelaceae) are a prolific source of secondary metabolites, predominantly producing sesquiterpenes, diterpenes, triterpenes, C_{15} acetogenins, acetylenes and chamigrenes (Erickson, 1983). *Laurencia* is a common genus occurring along southern Australian coasts, with numerous species being found in this region (König and Wright, 1997). The search for bioactive natural products from this genus of red algae has been an active area of research since the early 1970s (Sims and Fenical, 1972) with the discovery of many interesting structural classes including C_{15} acetogenins, such as laurendecumenyne A (**1**) (Nai-Yun et al., 2007) and pannosallene (**2**) (Suzuki et al., 1996). Many of the chamigrenes reported from the genus are halogenated and contain a *spiro* centre, along with cyclohexane moieties such as ma'ilione (**3**) (Francisco and Erickson, 2001) and the sesquiterpene bromo diether (**4**) (Kikuchi et al., 1985).

As part of the activities of the Marine And Terrestrial NAtural Product (MATNAP) research group at RMIT University, which studies the chemistry and biological activity of southern Australian marine organisms, we examined a specimen of the red alga, *Laurencia elata* collected from St. Paul's Beach, Sorrento, Victoria, Australia. The crude extract of the alga displayed slight antitumour activity as well as antifungal and antiviral activities. We describe herein the chemical profiling strategy employed (on-flow and stop-flow HPLC–NMR) to preliminary examine the crude extract of the specimen, together with the subsequent chemical investigation that led to the isolation and structure determination of two C_{16} chamigrenes, cycloelatanene A (**23**) and cycloelatanene B (**28**). This investigation also resulted in the isolation of several previously described compounds including (3*Z*)-chlorofucin (**5**), pacifenol (**8**) and elatenyne (**10**). 1D NOE NMR spectroscopy was utilised to assign the relative configuration of cycloelatanene A (**23**) and cycloelatanene B (**28**). In addition, an attempt to address the relative configuration for the recently revised structure of elatenyne (**10**) was also undertaken.

2. Results and discussion

2.1. Extraction of L. elata for on-flow HPLC–NMR profiling

An initial small-scale DCM extraction of the alga was undertaken according to the procedure outlined in Section 4.1.4. The intention was to employ HPLC–NMR to quickly profile the class of secondary metabolites present in the crude extract as well as to attempt to identify the presence of possible previously unrecognised constituents.

Initial chemical profiling of the *L. elata* crude extract in CH_3CN employing on-flow HPLC–NMR analysis resulted in a WET1D ^1H

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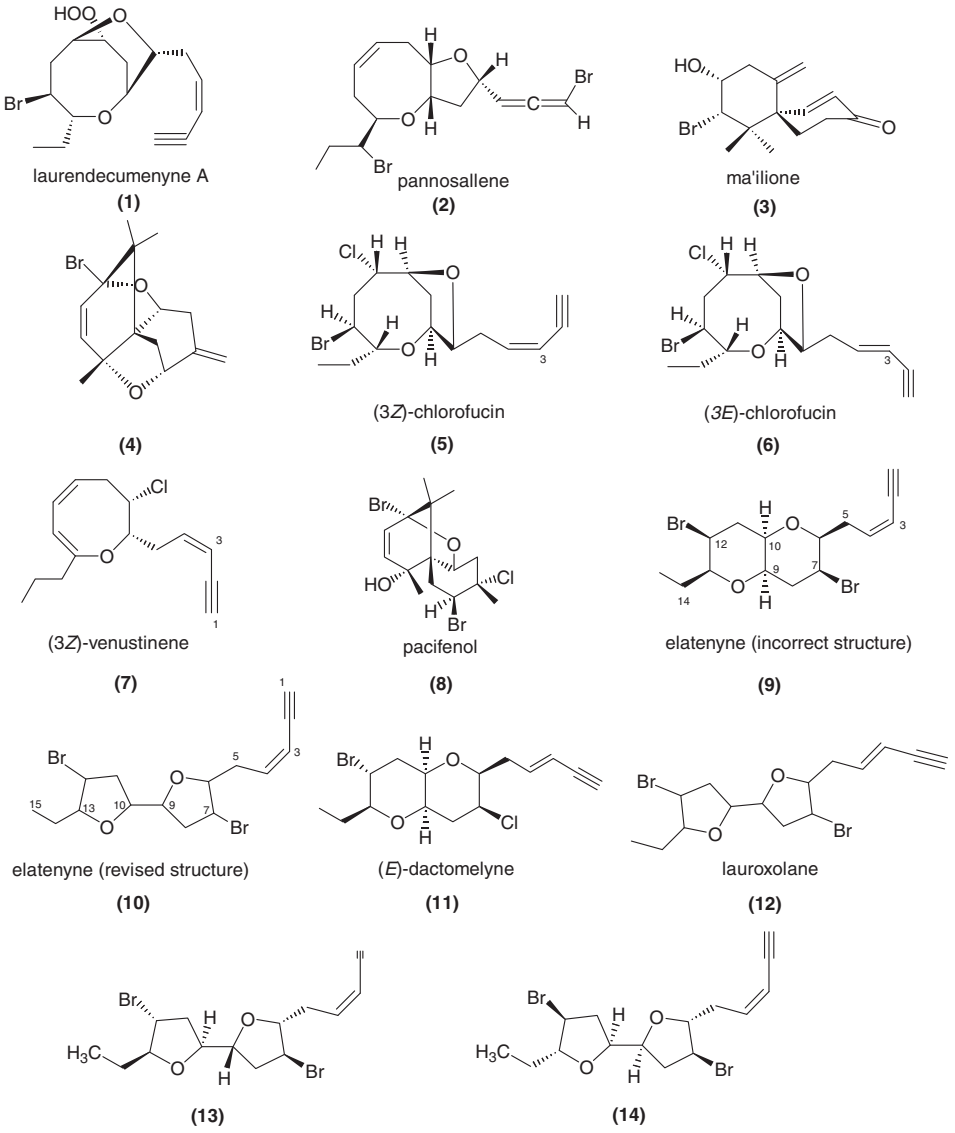

NMR spectrum that suggested the presence of halogenated terpenes, which represent a class of compound typical of this genus (Fig. 1). Subsequent stop-flow HPLC–NMR allowed for the individual WET1D ^{1}H NMR spectra of the major metabolites to be obtained (Fig. 1). This enabled a partial identification of three of the secondary metabolites (**5**, **8** and **10**). In all cases significant signal suppression was observed and it was concluded that an off-line isolation and complete structure determination of these compounds would be necessary to permit their unequivocal identification. A combined chemical profiling approach was adopted that involved both on-line (HPLC–NMR analysis) and off-line (HPLC isolation and subsequent NMR and MS) studies to confirm and establish the nature of the principle components in the crude extract.

2.2. Extraction of *L. elata* for larger scale off-line chemical investigation

A large-scale extraction of the marine alga, *L. elata* was undertaken according to the procedure outlined in Section 4.1.3.

(3*Z*)-chlorofucin (**5**) was isolated as an unstable yellow oil. The positive mode ESIMS displayed a *m/z* value of 369 $[M+Na]^+$, which supported a molecular formula of $C_{15}H_{20}BrClO_2$. The structure of **5** was confirmed by a direct comparison of the proton and ^{13}C NMR data with those in the literature (Suzuki et al., 2001; Suzuki, 1980). The corresponding isomer (3*E*)-chlorofucin (**6**) had been previously isolated from the marine algae, *Laurencia snyderae* Dawson (Howard et al., 1980), *Laurencia pannosa* (Suzuki et al., 1996) and also from a Malaysian species of *Laurencia* (Vairappan et al., 2008). A comparison of the NMR data of (**5**) and (**6**), together with a comparison of the proton ^3J coupling constants for the $\Delta^{3,4}$ double bond [(δ_H 5.55, *dt*, *J* = 2.5, 11.0 Hz) and (δ_H 6.03, *ddd*, *J* = 7.5, 8.0, 10.5 Hz)] in (**5**) to those of (3*Z*)-venustinene (**7**) [(δ_H 5.55, *brd*, *J* = 11.0 Hz) and (δ_H 6.00, *ddd*, *J* = 7.0, 11.0, 11.0 Hz)] (Suzuki et al., 1983) confirmed **5** to be the (3*Z*)-isomer of chlorofucin.

Pacifenol (**8**) was isolated as stable colourless needles. The ESIMS showed the presence of a peak at *m/z* 464 $[M+Cl]^-$, consistent with a molecular formula of $C_{15}H_{21}Br_2ClO_2$. The 1D and 2D spectroscopic NMR data and the mass spectrum were found to be identical to those previously reported (Argandoña et al., 1993; Kaiser et al., 2001). Pacifenol (**8**) is a sesquiterpene which contains a *spiro*[5.5]undecane skeleton and was first isolated from the red alga, *L. pacifica* and previously characterised by spectroscopic methods as well as by single-crystal X-ray analysis (Sims et al., 1971). Pacifenol (**8**) has been reported from a number of marine organisms including: *Laurencia nidifica* (Kimura et al., 1999; Waraszkiewicz and Erickson, 1974), *Laurencia nipponica* Yamada (Suzuki, 1980), *Laurencia claviformis* (Argandoña et al., 1993), *Laurencia majuscula* (Caccamese et al., 1986) and from the Brazilian mollusc *Aplysia dactilomela* (Kaiser et al., 2001).